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Article

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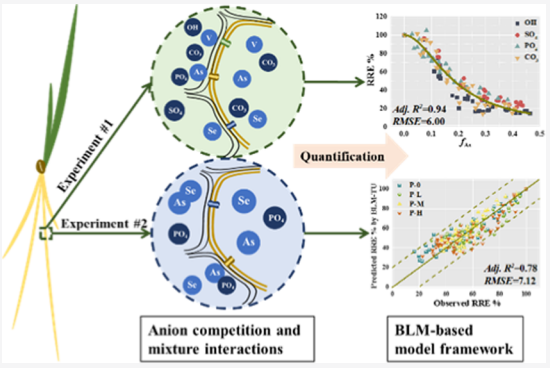
ABSTRACT: Hardly any study has focused on the quantitative modeling of the toxicity of anionic metal(loid)s and their mixtures in the presence of potentially competing anions. Here, we designed a univariate experiment (420 treatments) to investigate the influence of various anions (phosphate, sulfate, carbonate, and OH^-) on the toxicity of single anionic metal(loid)s (arsenate, selenite, and vanadate) and a full factorial mixture experiment (196 treatments) to examine the interactions and toxicity of As−Se mixtures at 4 phosphate levels. Standard root elongation tests with wheat (*Triticum aestivum*) were performed. A modeling framework, resembling the biotic ligand model (BLM) for cationic metals, was developed, extended, and applied to explain anion competitions and mixture effects. Carbonate significantly alleviated the toxicity of all three metal(loid)s. The toxicity of As was significantly mitigated by phosphate, while V toxicity was significantly relieved by OH^-. The BLM-like model successfully explained more than 93% of the observed variance in toxicity. With the parameters derived from single-metal(loid) exposures, the developed BLM-toxic unit model reached an overall prediction performance of 78% in modeling the toxicity of As−Se mixtures at varying phosphate levels, validating the effectiveness of the model framework. It is concluded that by taking possible anion competitions and interactions into account, the BLM-type approaches can serve as promising tools for the risk assessment of single and mixed metal(loid)s contamination.



INTRODUCTION

A considerable part of metal(loid)s exist as anions in the natural world. Despite their indispensable role in the environment, interactions between anions have not systematically been taken into consideration by traditional models for bioavailability prediction as is the case for cations. Based on the fact that the root plasma membrane surface is negatively charged,[1] the electrostatic model assumes that anions are repelled from plasma membrane surfaces. Anions thus have a negligible impact on the surface electrical potential, which determines the phytotoxicity of ions.[2,3] Similarly, anion interactions are not fully discussed in the theory behind the biotic ligand model (BLM). The BLM has been constructed based on the findings that the free cation activity is a more accurate predictor of metal bioavailability than the dissolved cation concentration.[4] Cation toxicity is, besides by chemical speciation, also influenced by the competitive binding to biotic ligands of other major cations, such as Ca^{2+}, Mg^{2+}, Na^+, or K^+.[5] Although the BLM has been developed into an extensively used tool to predict metal bioavailability in both aquatic and terrestrial systems,[6,7] very few studies have assessed its application to anionic metals.

Arsenic (As), selenium (Se), and vanadium (V) are ubiquitous anionic metal(loid)s worldwide. Known as a notorious contaminant, arsenic may cause health issues once it enters the food chain.[8] With a global mean concentration of 5 mg/kg in soil and a concentration range of up to 171 μg/L in the soil solution in Europe,[9] arsenic contaminates soil mainly through anthropogenic activities, including various metal mining and smelting activities and release of pesticides and wood preservatives.[10] Densely populated regions, especially Southern Asia, are at a high risk of arsenic exposure due to As-contaminated drinking water and groundwater, originating from natural sources.[11] Unlike arsenic, selenium is required as a trace element for the metabolism of many life forms from microalgae to mammals.[12] The Se concentration in soils worldwide generally ranges from 0.05 to 1.5 mg/kg, depending on soil properties.[13] The two major bioavailable species of Se in the environment are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), either of which can be the dominant species in oxidizing or reducing environments.[14] A concentration range of 0.51−27.64 μg Se/L in the soil solution was reported in

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Middle China.[15] Vanadium is also a potentially harmful metal to the environment with public concern. With an estimated average content of 97 mg/kg in the upper Earth crust,[16] vanadium is the most abundant metal(loid) compared to arsenic and selenium. Though the typical vanadium concentration in the European soil solution was up to 0.1 mg V/L,[17] a vanadium content as high as 4794 mg/kg has been reported for V-contaminated soils from mining and smelting areas in Southwest China.[18]

The bioavailability and toxicity of metal(loid)s or anionic metals are considerably influenced by coexisting competing anions. Due to their chemical similarity to phosphate, both arsenate and vanadate can interfere with P-containing metabolic processes.[16,19] For example, arsenic is able to cross the root plasma membrane through inorganic phosphate (Pi) transporters and replaces Pi in the formation of ATP.[20] Correspondingly, phosphate has been demonstrated to influence the toxicity of arsenate and vanadate by competing for uptake sites.[21,22] A similar behavior was observed for selenium and its analogue sulfur. Selenate tends to be transported via sulfate transporters and channels, while selenite uptake is also mediated by phosphate transporter in plant.[23] A deficiency of sulfur increased the toxicity of selenium to the green alga.[24] In addition, adequate sulfur supply also mitigated arsenic toxicity to rice through several metabolic pathways.[25] As another important anion in soil solution, carbonate has been found to alleviate arsenic toxicity due to its buffering system and impact on the H^+ balance.[26,27] Nevertheless, a systematic investigation into the anion factors affecting toxicity is still lacking.

Apart from assessing the influence of competing anions, it is also of environmental significance to study interactions between anionic metal(loid)s since in nature metals tend to exist as mixtures.[28,29] Listed next to arsenic in the periodic table, with similar chemical properties, selenium is regarded as a required micronutrient at a controlled dose, while arsenic poses a significant risk to ecosystems. Interactions between arsenic and selenium have been extensively studied for humans, animals, and cells.[30] Although mostly antagonistic effects upon plants have been reported for As−Se mixtures,[30,31] synergistic interaction was observed in the alga *Desmodesmus quadricauda*.[32] These inconsistent conclusions were mainly derived from the experiments with a parsimonious design of mixture concentration combinations. In addition, the impact of exposure conditions on mixture interactions and toxicity is often ignored. This makes it difficult to interpret and reconcile As−Se mixture effects from different studies.

The interplay between anions or anionic metal(loid)s needs to be quantitatively taken into consideration to establish risk-based guidelines for metal(loid)s. Although the BLM has been developed mainly for cationic metals and mixtures of cations, its mathematical frameworks may offer practical reference also for predicting the bioavailability of anionic metal(loid)s, even in co-contaminated systems. In a recent study, the potential of the BLM to predict soil arsenic toxicity has been demonstrated.[26] The authors reported that porewater carbonate and phosphate can be used as potential predictors of arsenate toxicity in soils. However, the identification or separation of these multiple toxicity-modifying effects in soils is usually based on empirical regression and therefore not transparent. This mainly results from the limitations inherent in soil tests, namely, the intercorrelations among soil variables (e.g., pH, phosphate, and carbonate). In contrast, hydroponic tests may be used to overcome this problem by univariately changing one of the covarying factors. More importantly, this would allow the derivation of mechanistic-based BLM parameters including binding constants of anions, which are currently unavailable. When it further comes to the prediction of mixture toxicity, the use of BLM-based approaches is feasible assuming different anions compete with each other for binding to the biotic ligands.[33] In our previous studies regarding cationic metal mixtures, the extended BLMs maintained relatively good performance and simplified the calculation of toxicity, especially under varying levels of competing ions.[34,35] It remains unknown whether the BLM-based approach is suitable for anionic metal(loid) mixtures and how the ways of model expansion affect its predictive ability.

The present study was therefore designed to quantify the bioavailability and toxicity of several anionic metal(loid)s (arsenate, selenite, and vanadate) in the presence of the potentially competing ions (phosphate, sulfate, carbonate, and OH^-) and further to model the possible nonadditive effects of As and Se mixtures under varying conditions. These three particular oxoanions of arsenate, selenite, and vanadate were selected because of their common tendency to utilize phosphate transporters as mentioned above.[16,22,25] By implementing 4-day acute toxicity assays for wheat (*Triticum aestivum*) in hydroponic culture, we attempted to identify: (1) to what extent the coexisting anions affect the phytotoxicity of anionic metal(loid)s, and whether a BLM-like model is a viable way to incorporate these toxicity-modifying factors; (2) whether the parameters derived from single-metal tests could be used to predict the toxicity of binary mixtures, thus greatly simplifying the calculation of toxicity of mixtures of metal(loid)s in practical applications; and (3) whether the nonadditive effects of arsenate and selenite at varying phosphate levels could be rationalized and predicted by an extended BLM-like model. The final goal was to establish a BLM-type bioavailability prediction model framework for both single and binary anionic metal(loid)s.

MATERIALS AND METHODS

Toxicity Bioassays. Wheat seeds (*T. aestivum*) were sterilized and germinated in a modified Hoagland solution (see Table S1 for details). The seedlings with root lengths of 1.0−1.5 cm were transferred to glass beakers filled with 250 mL of a test solution. Parafilm traps were used to fix four seedlings in each beaker and keep the roots immersed in the solution. Beakers were wrapped with aluminum foil. Three replicates were set for each treatment. Then, the 4-day wheat toxicity assays were conducted in a phytotron at 20 °C, 75% humidity, and a light/dark cycle of 16:8 h. The test solutions were renewed daily to avoid potential redox changes and to maintain the desired solution composition.

After exposure, the longest root length of each plant was measured. Control groups were set respectively for each concentration of factors (pH, sulfate, phosphate, and carbonate). Relative root elongation (RRE, % of control) was used as the toxicity endpoint and calculated as follows

$$\mathrm{RRE}(\%) = \frac{\mathrm{RE_A}}{\mathrm{RE_C}} \times 100\% \quad (1)$$

where RE_A is the average of the longest root lengths (cm) of the four exposed plants and RE_C is the average of the longest root lengths (cm) of the control plants.

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Exposure Treatments. Twelve sets of univariate single-metal(loid) experiments were conducted, including four factors (pH, sulfate, phosphate, and carbonate) for three metal(loid)s (As, Se, and V), and four sets of binary tests were conducted for As−Se mixtures with four phosphate concentrations (Table S2). Stock solutions of arsenate, selenite, vanadate, sulfate, phosphate, and carbonate were prepared using $Na_2HAsO_4{\cdot}7H_2O$, $Na_2SeO_3{\cdot}5H_2O$, $NaVO_3$, Na_2SO_4, Na_3PO_4, and Na_2CO_3, respectively. Sodium salts were used because a preliminary study showed that the influence of Na^+ on the toxicity of three tested metal(loid)s was negligible (Figure S1). Sodium metavanadate ($NaVO_3$) was used because in water it transforms quickly into orthovanadate (Na_3VO_4) with less changes in salinity and pH than orthovanadate itself.[36] To obtain test solutions for the different treatments, the stock solutions were diluted with nutrient solutions, which contained 0.2 mM $CaCl_2$ and 5 μM H_3BO_3 (see Table S2 for details). All test solutions were buffered at approximately pH 6.0 using diluted HCl and 0.75 mg/L 2-(*N*-morpholino)-ethanesulfonic acid (MES), except for the pH (OH^-) sets, which were adjusted to pH 4.8, 5.4, 6.0, 6.6, 7.2, and 8.0 using diluted HCl, diluted NaOH, and 0.75 mg/L MES (active in the pH range of 5.5−6.7) or 0.75 mg/L 3-(*N*-morpholino)-propanesulfonic acid (MOPS) (active in the pH range of 6.5−7.9).

Chemical Measurements and Speciation Calculations. The actual dissolved concentrations of As, Se, V, P, and coexisting cations (Ca^{2+}, Na^+) in the test solutions before exposure were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES, iCAP7600, Thermo Fisher). The sulfate concentrations were determined using ion chromatography (MIC, Metrohm), and the carbonate concentrations were measured by a total organic carbon (TOC) analyzer with a nondispersive infrared sensor (multi 3100, Analytik Jena). All dissolved concentrations were expressed as $[M]_{diss}$, where M = As, Se, or V.

The pH of the test solutions was measured by a pH meter (Delta 320, Mettler Toledo) before and after exposure. The average pH values were input for the chemical speciation calculations with the software Visual MINTEQ (database of version 4.0), along with temperature (20 °C) and dissolved concentrations of the components (Table S3). At pH values around 6 in the wheat assays, the dominant species were $H_2AsO_4^-$ (78.0−97.6%), $HSeO_3^-$ (98.3−99.7%), $H_2VO_4^-$ (99.1−99.9%), $H_2PO_4^-$ (90.2−98.7%), along with both HCO_3^- (15.3−75.3%) and H_2CO_3 (24.0−84.7%) for carbonate (Figure S2). The anion activities of selenite, vanadate, phosphate, and carbonate are presented as the activities of $H_2AsO_4^-$, $HSeO_3^-$, $H_2VO_4^-$, $H_2PO_4^-$, and HCO_3^-, expressed as $\{H_2AsO_4^-\}$, $\{HSeO_3^-\}$, $\{H_2VO_4^-\}$, $\{H_2PO_4^-\}$, and $\{HCO_3^-\}$, respectively.

Data Analysis. The mathematical BLM framework was adapted to determine the impact of competing anions on the phytotoxicity of anionic metals. In the BLM, the toxicity can be predicted as the fraction of metal-occupied biotic ligands (BL)[6]

$$f_M = \frac{K_M{\cdot}\{M\}}{1 + K_M{\cdot}\{M\} + \sum K_A{\cdot}\{A\}} \quad (2)$$

where f_M is the fraction of BL occupied by As, Se, or V; K_M is the binding constant of the metal(loid) to the BL; K_A is the binding constant of a competing anion (A); and {} represents anion activities.

A log-logistic function was used to fit the dose−response curves of As, Se, and V in Origin 9.0, based on three dose expressions

$$\mathrm{RRE}(\%) = \frac{100}{1 + (x_i/x_i^{50})^{\beta}} \quad (3)$$

where x_i is the dissolved concentration, activity, or BL fraction of the three metal(loid)s; x_i^{50} is the dose causing a 50% reduction of RRE compared to the control; and β is the slope factor.

In the present study, the As−Se mixture was used to verify the performance of BLM-based models in the prediction of binary mixture toxicity. Two ways of model expansion, on the basis of different assumptions, were adopted. Toxic unit (TU) approaches are based on noncompetitive binding due to diverse mechanisms.[34] Here, a TU-combined BLM was applied to explain the toxicity of As−Se mixtures at different phosphate levels

$$\mathrm{TU} = \sum_{i=1}^{n} \mathrm{TU}_i = \frac{c_{As}}{\mathrm{EC}x_{As}} + \frac{c_{Se}}{\mathrm{EC}x_{Se}} \quad (4)$$

where c_{As} and c_{Se} are the activities of As and Se, respectively, and $\mathrm{EC}x_{As}$ and $\mathrm{EC}x_{Se}$ are the effective activities at which a x % toxic response is observed.

To combine BLM and TU approaches, eqs 2 and 4 can be transformed

$$f_{As} = \frac{K_{As}{\cdot}\{As\}}{1 + K_{As}{\cdot}\{As\} + \sum K_A{\cdot}\{A\}} \quad (5)$$

$$f_{Se} = \frac{K_{Se}{\cdot}\{Se\}}{1 + K_{Se}{\cdot}\{Se\} + \sum K_A{\cdot}\{A\}} \quad (6)$$

$$\mathrm{TU} = \frac{f_{As}}{f_{50,As}} + \frac{f_{Se}}{f_{50,Se}} \quad (7)$$

where f_{50} is the fraction of occupied BL where a 50% response is observed. Accordingly, TU_{50} is the toxic unit at a 50% response. Considering that strict additivity (TU = 1) rarely occurs, a deviation of 10% was allowed during the calculations.[37] Therefore, a TU value over 1.1 indicated antagonism in the present study.

For comparison, a modified BLM for mixtures was employed based on the assumption that both competition between anionic metals and between anions exists due to similar mechanisms of action[34]

$$f_{As} = \frac{K_{As}{\cdot}\{As\}}{1 + K_{As}{\cdot}\{As\} + K_{Se}{\cdot}\{Se\} + \sum K_A{\cdot}\{A\}} \quad (8)$$

$$f_{Se} = \frac{K_{Se}{\cdot}\{Se\}}{1 + K_{Se}{\cdot}\{Se\} + K_{As}{\cdot}\{As\} + \sum K_A{\cdot}\{A\}} \quad (9)$$

$$f_{mix} = f_{As} + f_{Se} \quad (10)$$

The model performance was evaluated by the Akaike information criterion (AIC). The AIC balances a model's goodness of fit with the consideration of the number of parameters, and the smallest AIC represents the best fit.[38] The AIC can be calculated as[39]

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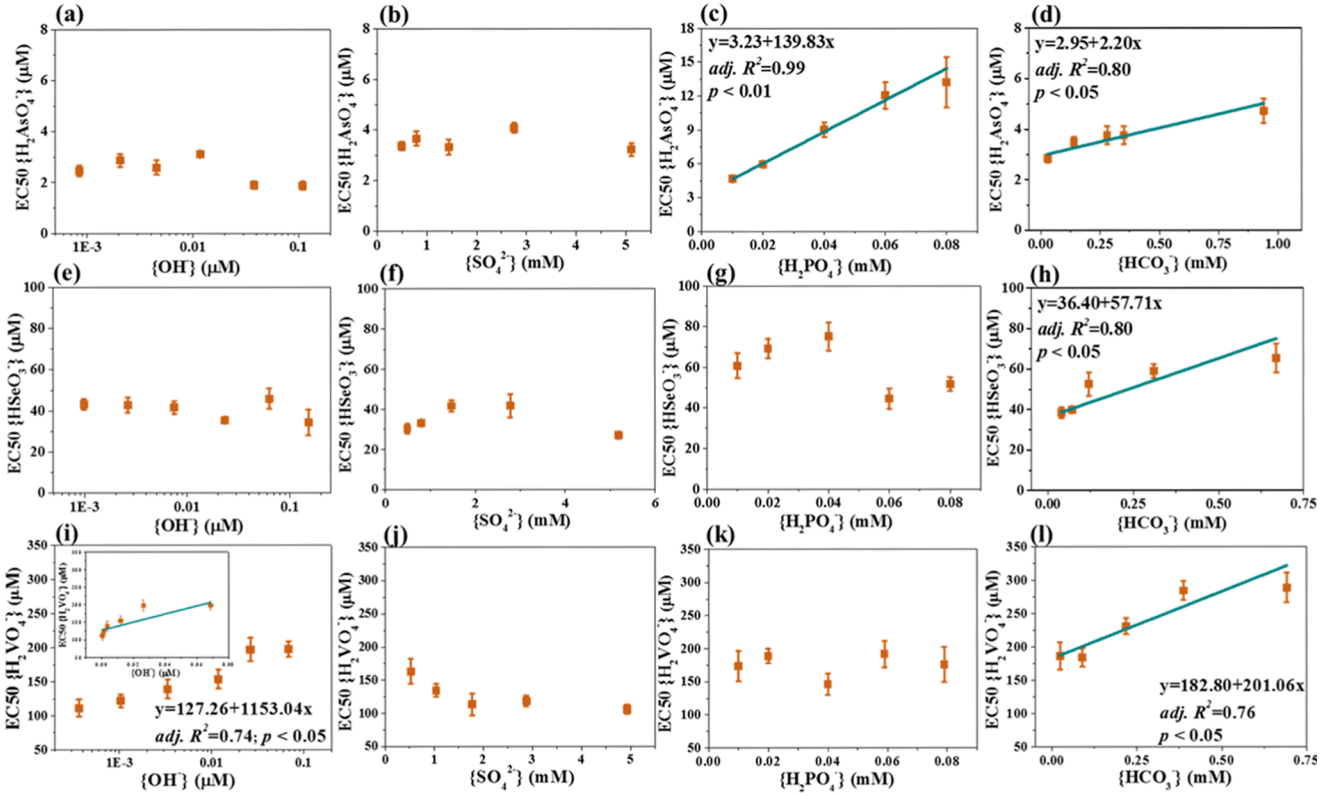

Figure 1. Relationships between median effective activities (EC50 values) for the toxicity of As (first row), Se (second row), and V (third row) to the root elongation of wheat (*T. aestivum*) after 4-day exposures and free-ion activities of OH^- (a, e, i), SO_4^{2-} (b, f, j), $H_2PO_4^-$ (c, g, k), and HCO_3^- (d, h, l). The solid lines represent statistically significant linear correlations ($p < 0.05$).

Table 1. Biotic Ligand-Based Model Parameters Derived from the 4-Day Toxicity of As, Se, and V to Wheat (*T. aestivum*) Root Elongation[a]

	binding constant (log K) (SE)					
metal(loid)s	OH^-	$H_2PO_4^-$	HCO_3^-	log K_M (SE)	f_{50} (SE)	β (SE)
As ($H_2AsO_4^-$)		4.69 (0.04)	2.98 (0.12)	4.91 (0.01)	0.18 (0.00)	1.91 (0.08)
Se ($HSeO_3^-$)				4.48 (0.01)	0.53 (0.01)	2.06 (0.09)
V ($H_2VO_4^-$)	6.61 (0.10)			4.06 (0.01)	0.57 (0.01)	3.32 (0.14)

[a] f_{50} = fraction of occupied biotic ligands at which 50% inhibition is observed; β = slope of the model; log K_M = binding constant; SE = standard error. All of the parameters were derived from a global fit, and K_{HCO_3} was applicable to all of the three metal(loid) anions.

$$\mathrm{AIC} = N \log\left(\frac{\mathrm{SS}}{N}\right) + 2n \tag{11}$$

where N is the sample size, SS is the residual sum of square, and n is the number of parameters.

The BLM-related parameters (conditional binding constants, β, f_{50}) were estimated using multiple nonlinear regression methods and obtained from a global fit based on all data in the single-metal(loid)s experiments. The regression modeling was performed using the SOLVER option in JMP 16.0 (SAS Institute) by minimizing the root-mean-square error (RMSE).[40]

RESULTS

Experiment 1: Anion Competition. As shown in Figure 1 and Table S4, quite different independent impacts of phosphate, sulfate, carbonate, and OH^- on the acute toxicity of As, Se, and V to wheat were observed. A statistically significant linear correlation between toxicity (EC50 values) and phosphate activities was observed for As only (Adj. R^2 = 0.99, p < 0.01). An increase in $\{H_2PO_4^-\}$ from 0.01 to 0.08 mM caused a 2.83-fold alleviation of As toxicity. $\{OH^-\}$ significantly affected V toxicity only (Adj. R^2 = 0.74, p < 0.05) with an increased $\{OH^-\}$ (3.6×10^{-4} to 0.069 μM) causing a 1.78-fold increase in EC50 values. An increased $\{HCO_3^-\}$ induced a significant (p < 0.05) decrease in the toxicity of As, Se, and V by 1.67-fold (Adj. R^2 = 0.80), 1.70-fold (Adj. R^2 = 0.80), and 1.55-fold (Adj. R^2 = 0.76), respectively. On the other hand, no significant linear correlations were observed between EC50 values and $\{SO_4^{2-}\}$ for all of the three metal(loid)s within the investigated concentration ranges.

Based on the results of the tests with the competing anions described above, the BLM-related parameters were derived (Table 1) from a global fit. The conditional binding constants equaled log $K_{H_2PO_4}$ = 4.69 for As, log K_{OH} = 6.61 for V, and log K_{HCO_3} = 2.98 for all of the three metal(loid)s. The estimated f_{50} values were 0.18, 0.53, and 0.57 for As, Se, and V, respectively.

Comparing the logistic fits of the RRE for the three metal(loid)s based on dissolved concentrations, free-ion

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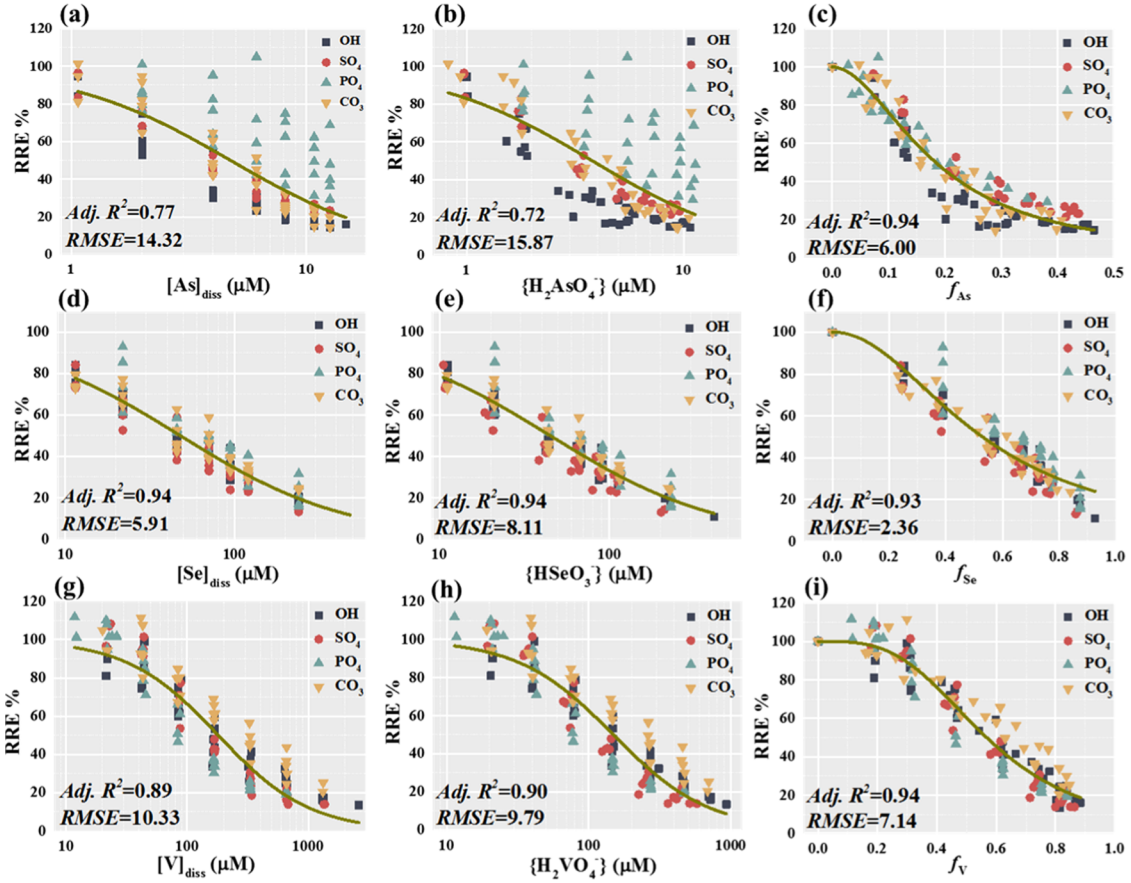

Figure 2. Dose−response relationships for the effect of As, Se, and V on relative root elongation (RRE, % of control) of wheat (*T. aestivum*) after 4-day exposure and related to three dose metrics, including dissolved concentrations (a, d, g), free-ion activities (b, e, h), and fractions of occupied biotic ligands (c, f, i). The solid lines represent the curves of logistic regression fit (eq 3) based on all data sets. RMSE indicates the root-mean-square error of the predicted RRE.

Table 2. Summary of Dose−Response Parameters and Coefficients of Toxicity Estimation for As, Se, and V on Wheat (*T. aestivum*) Root Elongation[a]

	$[M]_{diss}$			{M}			f_M		
	EC50 (μM) ± SE	Adj. R^2	RMSE	EC50 (μM) ± SE	Adj. R^2	RMSE	EC50 (μM) ± SE	Adj. R^2	RMSE
As	4.76 ± 0.24	0.77	14.32	3.79 ± 0.22	0.72	15.87	0.18 ± 0.00	0.94	6.00
Se	47.8 ± 1.39	0.94	5.91	45.0 ± 1.36	0.94	8.11	0.53 ± 0.01	0.93	2.36
V	180 ± 1	0.89	10.33	149 ± 6	0.90	9.79	0.57 ± 0.01	0.94	7.14

[a] $[M]_{diss}$ = parameters based on dissolved concentrations; {M} = parameters based on free-ion activities; f_M = parameters based on fractions of occupied biotic ligands; SE = standard error.

activities, and biotic ligand fractions (Figure 2, Table 2), the greatest improvement of Adj. R^2 (from 0.77 and 0.72 to 0.95) was observed in the case of competition testing with As, and the corresponding RMSE was reduced from 14.32 and 15.87 to 6.00. A small increase of Adj. R^2, from 0.89 to 0.94, was found in the V test sets, with RMSE decreasing from 10.33 and 9.79 to 7.14. Similar Adj. R^2 values of 0.94 and 0.93 were derived for the Se sets despite a decrease of RMSE from 5.91 and 8.11 to 2.36. To be specific, the BLM-based model reached an improved model performance by incorporating respective competing anions of the three metal(loid)s (Figure S3).

Using a BLM-based model to predict metal(loid) toxicity, both the lowest f_{50} value and the highest binding constant K_M were obtained for As, indicating the highest toxicity and the strongest binding affinity to the biotic ligands (Table 1). The highest f_{50} value and the lowest K_M were derived for V (Table 1). This was in accordance with the ranking of EC50 values based on both dissolved concentrations and activities (Table 2). It may therefore be concluded that model predictions were established as based on rational model parameters and coefficients.

Experiment 2: Metal(loid)s Interactions. Binding constants of arsenate, selenite, and phosphate obtained in experiment 1 (Table 1) were used to evaluate the results of the As−Se mixture toxicity assays. In the prediction of the toxicity of As−Se mixtures (Figure 3), the BLM-TU model (Adj. R^2 = 0.78) reached a significantly higher precision level with RMSE of 7.12 (Figure 3b) than the BLM-f_{mix} model (Adj. R^2 = 0.41, RMSE = 14.85, Figure 3a).

In the BLM-TU models, synergism was observed at high toxic effect levels (Figure 3b). Antagonism was, on the other hand, observed at low toxic effects, with an overlapping range

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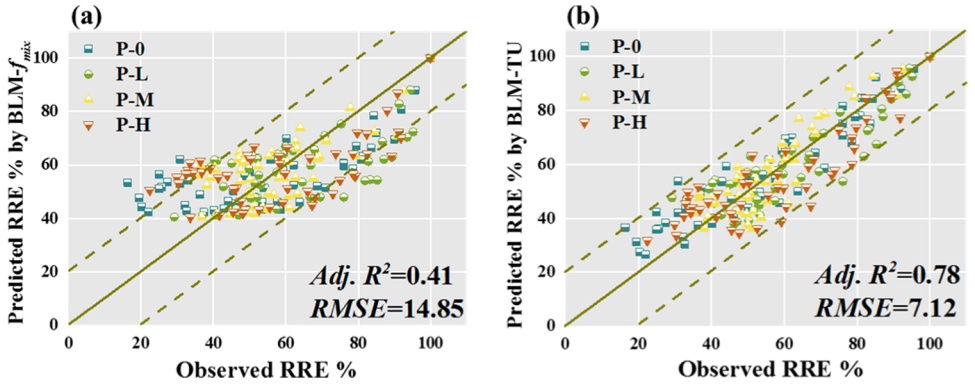

Figure 3. Relationships between predicted and observed relative root elongation (RRE, % of control) of wheat (*T. aestivum*) after 4-day exposure to As−Se mixtures at dissolved phosphate concentrations of 0 μM (P-0), 45 μM (P-L), 91 μM (P-M), and 185 μM (P-H). The relative root elongations were predicted using a BLM-f_{mix} model (a) and a BLM-TU model (b). The solid lines represent the perfect 1:1 fit lines. In the BLM-TU model, the dots below the line indicate potential antagonistic interactions and the dots above indicate potential synergistic interactions. The dashed lines represent a 20% difference between the predicted and observed RRE. Adj. R^2 indicates adjusted coefficient of determination based on all data sets.

of 40−60% observed RRE (Figure 3b). To be specific, the BLM-TU model improved the model performance in the lower and higher ends of the RRE range, compared to the BLM-f_{mix} model. Accordingly, the BLM-TU model obtained a comprehensively better model performance with a smaller AIC value of 402.6 than that of the BLM-f_{mix} model (AIC = 488.0) (Table S5). The overall toxicity of binary As−Se mixtures at varying phosphate levels was reasonably well predicted by the BLM-TU model (Figure 3b).

The prediction parameters of the two approaches at dissolved phosphate concentrations of 0 μM (P-0), 45 μM (P-L), 91 μM (P-M), and 185 μM (P-H) are shown in Table 3. The highest R^2 values (0.50 and 0.87, respectively) were obtained at P-0. In the presence of phosphate, the P-M sets (91 μM) had the highest precision levels in each model (R^2 = 0.43 and 0.80) and the lowest RMSE of 11.50 and 7.57, respectively.

Table 3. Summary and Parameters of Toxicity Modeling the Toxicity of As−Se Mixtures to Wheat (*T. aestivum*) Root Elongation at Different Phosphate Levels Using BLM-f_{mix} and BLM-TU Models[a]

	BLM-f_{mix}		BLM-TU	
	R^2	RMSE	R^2	RMSE
P-0	0.50	16.65	0.87	8.94
P-L	0.42	14.31	0.77	8.90
P-M	0.43	11.50	0.80	7.57
P-H	0.33	16.35	0.75	10.24

[a]P-0, P-L, P-M, and P-H = dissolved phosphate concentrations of 0, 45, 91, and 185 μM, respectively.

DISCUSSION

Dependent Influence of Phosphate. The increase of phosphate activities from 10 to 80 μM induced a statistically significant increase of EC50 values for arsenate toxicity to wheat elongation (Figure 1c). The inhibition of *Microcystis aeruginosa* growth by arsenate was found to be mitigated by increasing phosphate supply (0−175 μM) in the culture medium.[22] Furthermore, suppression of phosphate transporters in As-tolerant barley was observed to be a mechanism to inhibit the uptake of arsenate.[41]

Although both arsenate and vanadate are chemical analogues to phosphate,[16,19] phosphate levels did not significantly correlate with vanadate toxicity in the same concentration range as arsenate. Imtiaz et al.[42] found that higher levels of phosphorus (500−1000 μM) significantly decreased vanadium uptake by chickpea genotypes in culture medium. We assume that the range of phosphate levels used in our study was relatively too small for vanadate, with an average EC50 value of 149 ± 6 μM (Table 2). To validate this assumption, the same wheat toxicity bioassays were conducted using an expanded range of phosphate concentrations of up to 600 μM. The results are shown in Figure S4. With Adj. R^2 = 0.90 and p < 0.01, the significant impact of phosphate on V toxicity was confirmed. However, because of environmental considerations, the data generated within the enlarged concentration range were not taken into consideration in modeling vanadate toxicity. After all, phosphorus concentrations in the soil solutions are generally low (0.001−0.4 mg P/L) due to quick and strong sorption to soil particles and do not exceed 5.0 mg/L (162 μM) even in fertilized soils.[43] In model establishment, we therefore decided to restrict data use to environmentally relevant concentration ranges.

Impact of OH^- (pH). Anion speciation is also, to a great extent, determined by pH. It has, for instance, been reported that CrO_4^{2-} gradually took over $HCrO_4^-$ with increasing pH values from 4.5 to 8.5 so that the consideration of both species was required when modeling Cr(VI) toxicity to barley.[44] Nevertheless, the dominant species remained the same ($H_2AsO_4^-$, $HSeO_3^-$, and $H_2VO_4^-$ for As, Se, and V, respectively) under the test conditions (Figure S2), and hence the model performance was not compromised using only the dominant species in the present study. Besides changing chemical species, it is obvious that pH may also affect anionic metal toxicity by offering OH^- as the competing anions. However, a statistically significant impact of OH^- was only observed in the vanadate assays (Figure 1i) but not in the arsenate (Figure 1a) and selenite assays (Figure 1e). This may be attributed to a more competitive and pH-dependent binding pattern of vanadate to the biotic ligands according to the assumptions of the BLM. Though $H_2VO_4^-$ remained the dominant species in the tests, vanadium, unlike arsenic and

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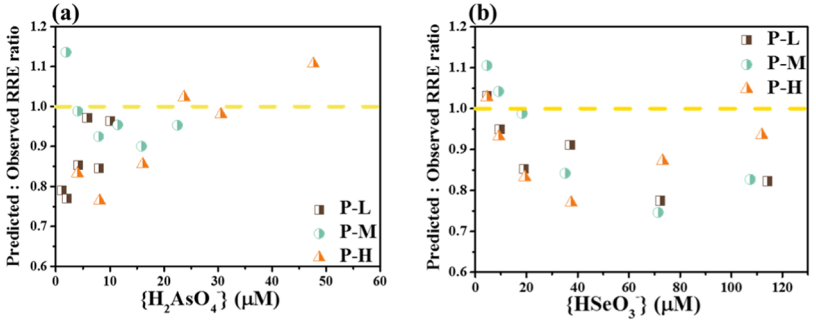

Figure 4. Relationships between the predicted-to-observed relative wheat (*T. aestivum*) root elongation ratio (RRE, % of control) as predicted by the combined BLM-TU model and $H_2AsO_4^-$ activities at a constant dissolved Se concentration of 19.84 μM (a), together with $HSeO_3^-$ activities at a constant dissolved As concentration of 4.50 μM (b), at dissolved phosphate concentrations of 0 μM (P-0), 45 μM (P-L), 91 μM (P-M), and 185 μM (P-H). The dashed lines represent ratios equal to 1 (for comparison only), below which the dots indicate potential antagonistic interactions and above which the dots indicate potential synergistic interactions.

selenium, was reported to form more complex species including oligomers like decavanadates, depending on pH and total vanadium concentration.[16] Since vanadate is more intrinsically susceptible to pH, OH^- may correspondingly compete with vanadate more effectively.

Role of Carbonate. As a competing anion, carbonate had a common statistically significant impact on the toxicity of As, Se, and V in the investigated concentration range. The change in pH values might account for some of the effects observed in the presence of varying carbonate concentrations. With both HCO_3^- and H_2CO_3 being dominant species in the test solutions, carbonate served as a buffer system to maintain the relatively high pH values (Table S3) despite the acidic root exudates.[45] Nevertheless, the significant impact of OH^-, which can be regarded as a dual verification for the assumption of pH changes, was only observed in V tests. Increasing pH (OH^-) and increasing carbonate activities (HCO_3^-–H_2CO_3 buffer system) can be mutually explained, both of which resulted in the alleviation of vanadate toxicity. This meanwhile indicated that different major competition mechanisms underlay the role of carbonate in As and Se toxicity alleviation.

Carbonate was found to decrease arsenate uptake by cucumber plants by drastically inhibiting the active H^+ co-transporters, which is necessary for its transport through Pi pathways.[26,27] Both arsenate and selenite can cross the plasma membrane surface of root cells via phosphate transporters,[20,23] while only arsenate toxicity to wheat was significantly affected by phosphate at the concentration range investigated in the present study. The theory of inhibition of phosphate transporters therefore was more evidently supported by the arsenate toxicity assays.

For selenite, the impact of carbonate might be the comprehensive outcome of anion competition and Pi transport inhibition, which cannot be directly reflected in the influence of OH^- and phosphate separately. Metal(loid) bioavailability is determined by multiple factors, one of which predominated in the case of arsenate and vanadate in the present study.

Modeling Metal(loid) Toxicity. Similar Adj. R^2 values were obtained based on dissolved concentrations and free-ion activities, demonstrating a limited influence of these dose metrics on metal(loid) toxicity (Figure 2). Meanwhile, the BLM-based model showed a satisfying performance for all three metal(loid)s studied, with the greatest improvement for As (Figure 2). Anion competition was able to quantitatively explain the phytotoxicity and bioavailability of the three tested anionic metal(loid)s to wheat. This also indicated that quoting only the dominant species ($H_2AsO_4^-$, $HSeO_3^-$, and $H_2VO_4^-$) was adequately fair for toxicity modeling.

Considering both the model fitness and the number of parameters, the AIC values (Table S5) proved the improved performance of the BLM-TU model (Adj. R^2 = 0.77) compared to the BLM-f_{mix} model (Adj. R^2 = 0.39). This indicated different dominant mechanisms of action on wheat root elongation for As and Se (Figure 3). No significant impact of phosphate on Se toxicity was observed (Figure 1g). This observation reduced the possibility that selenite entered the plasma membrane of the wheat roots in the same way as arsenate by using phosphate transporters in the present study. Evidence has shown that selenite uptake of rice (*Oryza sativa*) was mediated by a silicon (Si) transporter.[46] This provides some insights into the diverse uptake and transport pathways. On the other hand, As–Se interactions may occur at an internal scale, which cannot be reflected in the current endpoints (RRE) or test organisms (wheat). As and Se have been reported to share similar methylation metabolic pathways and mutually inhibit excretions and damages, including reactive oxygen species (ROS) generation.[47] Kamo et al.[48] proposed that the nonadditive effects may be ascribed to a combination of processes of metal kinetics (metal speciation and binding to BLs) instead of the complex interaction mechanisms. In this case, mixture toxicity can be predicted with a simpler version of the biotic ligand model.

For the As–Se mixtures, slight synergism and antagonism were observed in the low and high range of RREs, respectively, when using the BLM-TU model (Figure 3b). The TU_{50} value obtained was 1.37 (more than 1.1), indicating an overall antagonistic effect. Studies have illustrated complex interactions between As and Se. An alleviative effect of Se on As toxicity was observed in rice grains[49] and Chinese brake fern (*Pteris vittata* L.).[31] Meanwhile, As–Se mixtures exerted more-than-additive toxicity to algae (*D. quadricauda*).[32] With most synergism and antagonism controlled within the 20% difference interval in the present study, the BLM-TU model succeeded in predicting 77% of the overall variations in As–Se mixture toxicity at all phosphate levels studied. The addition of phosphate induced additional alleviation, which complicates the assessment of the interactions between As and Se. In our study, various phosphate concentrations were incorporated using the model parameter $K_{H_2PO_4}$ in the calculation. For conventional toxicity models, such as concentration addition (CA) or independent action (IA),[50] the EC50 value of each

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single contaminant at every background level of competing ions is needed for the precise prediction of mixture toxicity. In other words, the extended BLM for mixtures of anionic metal(loid)s is a promising tool, especially in dealing with multianion scenarios.

Nonadditive Effects of Mixtures of As−Se with Phosphate. To elucidate the impact of phosphate on the interactions between As and Se, we chose a fixed Se concentration (19.88 μM) and a fixed As concentration (4.50 μM) for the comparison of predicted-to-observed RRE ratios (Figure 4). If the synergism and antagonism were strictly defined in accordance with the 20% deviation in Figure 3 (ratios below 0.8 or over 1.2), then only antagonism was observed under certain conditions. Nevertheless, the tendency of ratio changes was diverse. The predicted-to-observed RRE ratios gradually increased with increasing As activities in P-L and P-H (Figure 4a). The P-M set showed a reverse trend from potential synergism at low As activities to approximately additive effects at higher As activities (Figure 4a). Due to the significant effect of phosphate on As toxicity only (Figure 1c,g), the higher level of phosphate mitigated the antagonism to a greater extent, while this mitigation gradually disappeared with increasing As activity. On the other hand, the trend that RRE ratios decreased from potential synergism to antagonism was intensified by increasing Se in a lower range of activities and contained in a higher range (Figure 4b). The reverse trend was larger at higher phosphate concentrations. Therefore, mixture toxicity was not necessarily alleviated by improved nutrient conditions, which requires further studies on specific interactions between anions.

Implications. In the present study, a BLM-based model and its extension were established to predict the bioavailability of both anionic metal(loid)s and binary mixtures in the presence of competing anions. Although the models focused on the three specific redox forms of metal(loid)s, the binding constants of the three oxyanions studied and the relevant conditional factors were obtained for future application; these binding constants may also be applicable for the prediction of metal(loid) mixture toxicity. Further research efforts are needed, on the one hand, to reveal molecular-level (internal) interaction mechanisms between anions and, on the other hand, to analyze and model anion interactions and mixture toxicity in more complex scenarios, e.g., field-contaminated soils. Quantitatively taking anion competition and interactions into consideration may facilitate the establishment of guidelines for the risk assessment of metal(loid)s, which are ubiquitous in the environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b06837.

Composition of the nutrient solution used for seed germination (Table S1); concentration sets used for the single-metal(loid) and binary mixture toxicity assays (Table S2); measured concentration sets of the single and binary metal(loid)s toxicity assays (Table S3); EC50 values of metal(loid)s at varying activities of factors (Table S4); fitting statistics and AIC values of the BLM-based models (Table S5); relationships between EC50 values for metal(loid) toxicity and Na^+ activities (Figure S1); chemical speciation of involved anions as a function of pH (Figure S2); dose−response relationships between metal(loid)s toxicity and biotic ligand fractions with significantly effective anions (Figure S3); and relationship between EC50 values of V and increased phosphate activities (Figure S4) (PDF)

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Notes

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